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## *EDITORIAL*

### THE SPIRIT OF CHRISTMAS

EACH day that passes is a witness to the scientists' prowess in the systematic and formulated accumulation of knowledge and his influence upon the way of life. It may seem strange in this age of scientific achievement, when our research workers have disclosed the structure, the nature and the quality of matter so that they can cast it asunder and cause its constituent elements to act in their service or for their destruction, that many learned men are turning their thoughts towards ethical ideas which are born of a benign philosophy and not of material things. From the earliest times knowledge was collected and accumulated for practical purposes. It was necessary to ascertain the best time for sowing and reaping, to measure and divide the land, to harvest, preserve and store food for man and beast and to identify and treat the diseases which might beset them.

The accumulation of such information was a form of primitive science, for science in its widest interpretation means the search for factual knowledge which may be stored and utilised for practical purposes in the service of man. It was the great Greek thinkers who first turned men's thoughts from purely material things. They realised that nature ran an orderly course which was directed by some power which could not be explained nor emulated by the art of man. The pure scientist in his search for material truth may degenerate into a pedant who cannot see beyond the edge of the wood, who collects and

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stores facts as a squirrel does nuts. On the other hand, the philosopher in his meandering of thought may stir up such a fog of speculation and doubt that the road may become uncertain and obscure and material progress be placed at a discount. If scientific progress be measured only by the yard-stick of material gain, if we hold the intellectual and cultural pursuit of knowledge, as practised by those of philosophical bent, to be of no account, then life will lose much of its humanism, its mellowness, its joy and charm. The happiness of man depends more upon the virtue of his moral code than upon his knowledge of the universe or his discovery of some erudite scientific fact. The great thinkers of the past were fully cognisant of this simple truth and the religions which they founded were based upon a moral code. Some of these religions were austere, some were born of fear and superstition, others drew their strength from the state and were ordered by the law, but the Christian religion took as its theme the quality of humility and of love and good fellowship between men. At this period of the year Christians celebrate the natal day of the Founder of their faith, and for a short while the trials and tribulations of the temporal world are laid aside and the spirit of good fellowship and amity is supreme. This is best exemplified in the family circle when the wanderers return to gather around the parental festive board. Good cheer and fellowship are the most potent dispellers of anxiety, suspicion and gloom. If the family spirit could be extended to the wider international sphere. all the year could be as Christmas and the world would rest in peace.

## *GENERAL ARTICLES*

# HIGH PROTEIN HUMAN FOOD FROM DESTOCKING SCRUB ANIMALS

By DR. I. MANN

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### The Problem

In East Africa, certain areas are heavily overstocked with concommitant devastation of grass and soil, while in other areas Africans are suffering from chronic deficiency of animal proteins. Some tribes have a special word to describe " meat hunger " as distinct from ordinary hunger, a specialisation which reflects an age-old problem. Three figures showing the data of three case histories are included in the text.

Where overstocking exists in one area and meat deficiency in another, the normal approach would be to de-stock the overgrazed area and supply with meat those who cannot breed enough livestock. Such a simple solution is not practicable; to transport beasts on foot is either uneconomic because of the enormous distances or impossible because of disease. Slaughtering, at the producing end and the transport of meat to the consumer, needs rapid transport and refrigeration, services lacking in primitive countries. Similarly, canned meat is too expensive for the African consumer, and unpopular.

Many destocking schemes have failed since only the strongest and fattest beasts are capable of reaching a distant market or packing station. African cattle owners are averse to selling such beasts.

### The People

With the destocking of the Samburu District in Kenya, an entirely new method was tried. The 30,000 Samburu are a nomadic tribe of Nilo-Hamites, related to the Masai, who inhabit 9,000 square miles to the south of Lake Rudolph. Their land has a low, unreliable, badly distributed rainfall. They own 300,000 head of cattle and probably as many sheep and goats. " Their tribal character is complex," to quote the District Commissioner's words. " They are vain to a degree, self-conscious and proud, yet they respond readily to friendliness and fair treatment. They are indolent but tough, taciturn but prone to self-induced hysteria. While rigidly conservative in the mass, the individual approach can and has led to great advances being accepted."

Because of lack of markets for poor stock and a natural unwillingness to kill sick, lame or emaciated animals, tens of thousands of cattle died every dry season. In view of these recurrent losses it was thought that an abattoir brought to the beasts might induce their owners to sell such poor stock.

* A paper read at the scientific conference on "The Medical, Agricultural and Veterinary Aspects of Food Production and Utilisation in East Africa" held in Kampala, Uganda, on March 24-26, 1954.

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## The Scheme

In 1951 the African Land Utilisation and Settlement Board (now the African Land Development Board) granted £5,000 for a pilot project designed by the Veterinary Department. This scheme was intended to absorb uneconomic cattle and convert them into stock-feed and dried meat. The Administration gave its encouragement, and the Samburu District Council their assistance. It was agreed that this district council should receive Sh.4s. from the buyer, the Veterinary Department, and Sh.2s. from the seller, the Samburu cattle owner, per beast.

The African Land Utilisation and Settlement Board granted a further £4,000 to the District Commissioner, Samburu, as a suspense trading account. The local livestock officer buys the cattle and issues slips for payment to the District Commissioner's representative, who attends sales. The proceeds of

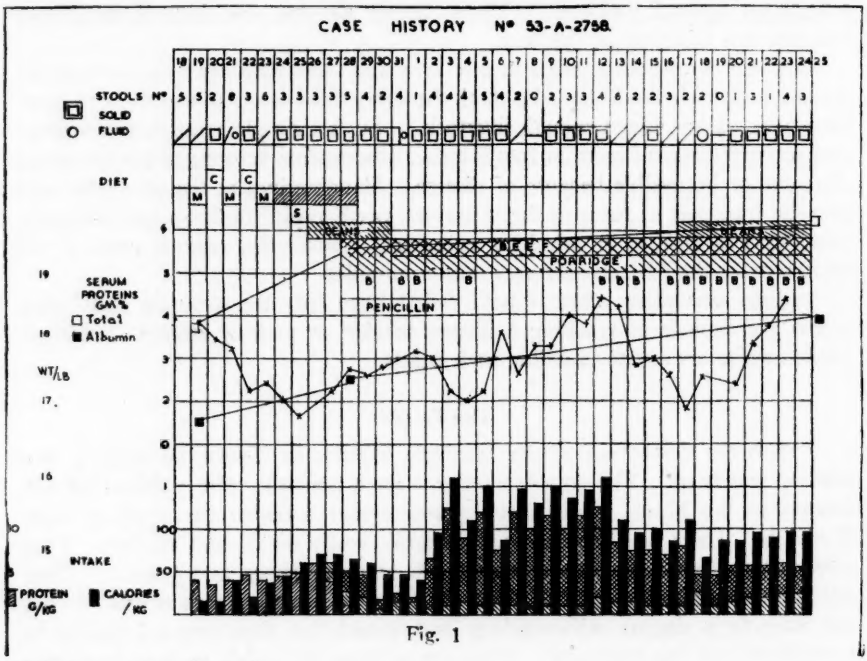


Fig. 1

*Makanga*— Age 22 months.

*History*— Born February, 1952. Breast fed—poor supply for 16 months supplemented with one bottle cow's milk daily for first 10 months then only tea and food. Illness started at time of weaning (16/12) with fever, œdema, diarrhœa and hair changes.

*Examination*—Very early skin change in groins and axillæ, raised pinkish patches. Hyperpigmentation over spine. Feel of skin quite good.
Œdema of feet and face.
Liver 0.
Spleen 0.
No marked anorexia. Took food well throughout.

*Progress*— As shown on chart.

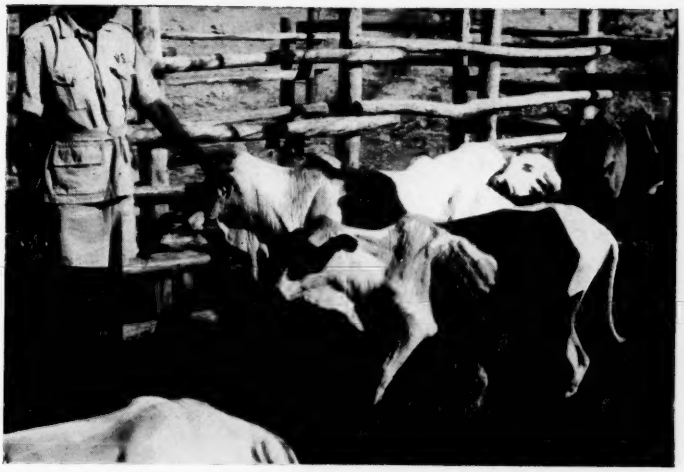

Fig. 1. Scrub cattle.

Fig. 2. The Abattoir.

Fig. 3. Humane killing.

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Fig. 4
Drying of meat.

Fig. 5
A forest of dried meat.

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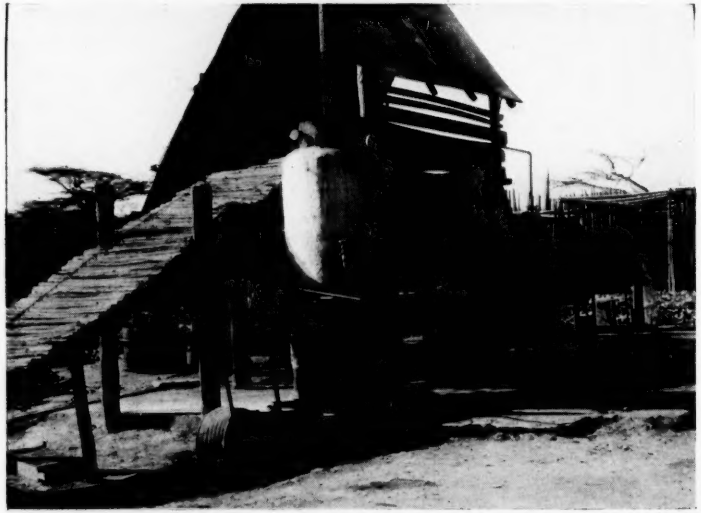

Fig. 6
Boiler and digestor.

Fig. 7
Blood drying.

Fig. 8
Filling tins with fat.

(*Article by Mann, page 491*)

Fig. 9
Samburu labourers.

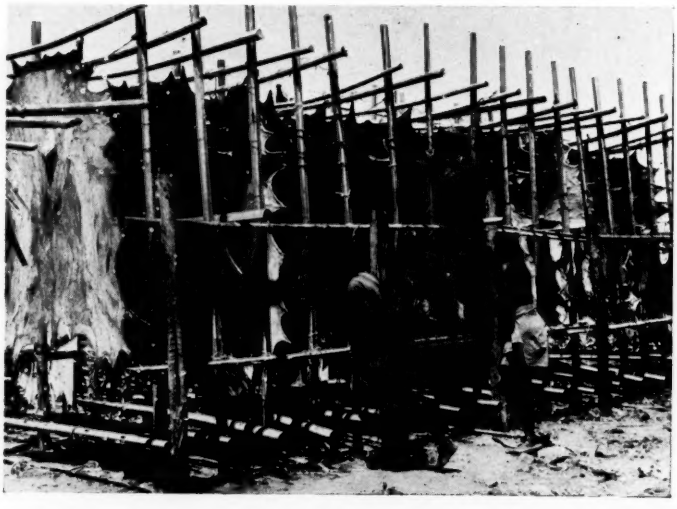

Fig. 10
Hide drying.

(*Article by Mann, page* 491)

the sale of abattoir products are credited to the District Commissioner's suspense account. This arrangement enables a good financial check.

High profits could not be expected from this scheme because of the many adverse factors such as the poor quality of the livestock, the distance from railhead, unskilled labour, the dependance on the weather, and the heavily branded hides. When the scheme was started three major problems remained to be answered:—

(*a*) Would sufficient cattle be offered?

(*b*) Could marketable products be made under bush conditions with untrained labour and primitive equipment?

(*c*) Could adequate local or overseas markets be found?

The Veterinary Department, who are responsible for destocking, pay the salary of the Livestock Officer in order not to burden the scheme with heavy overheads.

A simple abattoir was built in the southern corner of the Samburu country, at Archer's Post. This site was chosen because it enjoyed almost continuous sunshine and dry winds, was adjacent to a good river and accessible by road. The buildings and equipment are simple and cheap. The climate has been used to assist the processing. Despite such simplicity of equipment, utilisation of the whole animal has been achieved and the following products marketed:—

*Edible:* Dried beef, refined fat.

*Inedible:* Suspension dried hides, bone meal, meat meal, liver meal, carcase meal, blood meal, hoof and horn meal, dried ruminal contents, tail switches.

Ruminal contents have been incorporated in poultry feeds. Their chemical composition is as follows:—

| | |
|---|---|
| Moisture ... ... ... | 9.5 per cent |
| Ash ... ... ... ... | 8.4 per cent |
| Crude protein ... ... | 13.9 per cent |
| Fibre ... ... ... ... | 27.5 per cent |
| Carbohydrate ... ... | 36.5 per cent |

thus closely resembling bran. Experiments are at present being conducted in Eritrea to find if ruminal contents can be used as locust bait, thus replacing bran sorely needed elsewhere.

The average monthly kill ranges from 1,000 to 1,200 head of cattle. There has been no difficulty at any time in selling these products, in fact requests from the Congo, Uganda and the countries around the Red Sea have had to be refused. The stock-feed, sterilised under pressure, is sold either to large stock-feed manufacturers such as Unga, Ltd., and Arrow Products, or direct to farmers.

### Camels

When some 10,000 head of cattle had been processed at the Archer's Post abattoir the Somali in the area asked the authorities to buy camels as well. Investigations have shown that camel meat tastes like beef and no difficulties have been met in selling all of it as dried meat. The yield of fat per camel

is high, from 35 to 60 lb. The hump fat is snow-white, has no objectionable odour, and is excellent shortening. The military headquarters in Nairobi, after carrying out a series of trials, have placed a permanent order for 18,000 lb. per month. Since the abattoir was working to capacity it was impossible to handle camels as well as cattle, hence a special camel abattoir was built alongside the original one.

### Veterinary Control

All animals are subject to ante- and post-mortem examination. Every animal showing signs of fever is segregated in a sick " boma " where blood slides and temperatures are taken and the animals closely watched. These precautions are primarily to guard against anthrax. " Measles " are definitely destroyed by desiccation.

Physiological emaciation which occurs so often in starved cattle, does not interfere with the quality of the finished product, although the shrinkage is

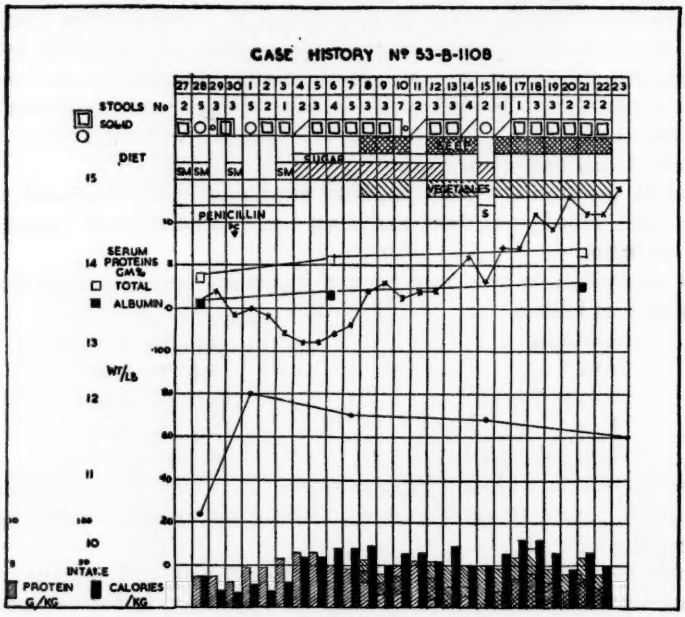


Fig. II

*Ziribagwa*— Age 14 months.

*History*— Born October 20, 1952. Breast fed, seven months weaned because of new pregnancy of the mother. Sent to grandmother and collected day before admission.

*Examination*—Œdema.
Hair soft and brown.
Skin: extensive raw area, vulva, groins, axillæ and back.
Angular Stomatitis.
Liver—not pathological.
Spleen 0.
Hookworm 0.

*Progress*— As shown on chart.

higher and the yield lower. Dried beef has proved extremely popular and supplies have never met the demand.

### Cost Per Animal

The cost of processing one beast is approximately Sh.9s. This sum includes the mortality encountered *en route* to the abattoir, the cost of slaughtering, the manufacture of all products, packing into containers and road transport 70 miles to railhead. Such low production costs and small capital outlay could not have been achieved in an orthodox meat-packing plant.

The cess of Sh.6s. per head was used by the African District Council to buy a tractor (to build dams), and to build a hospital, the finest building in the district.

### Dried beef

The dried meat has been analysed by the Medical Research Laboratory, Nairobi. Their analysis for beef from native cattle is given for comparison:—

| | Water | Protein | Fat | Ash |
|---|---|---|---|---|
| Native beef ... | 66 | 20.3 | 7.3 | 1.0 |
| Dried beef ... | 13.62 | 66.58 | 10.04 | 3.58 |

Dried beef has the following advantages:—

(1) One pound of fresh meat actually yields only 12 oz. after the bone has been removed, whereas dried beef being boneless is all edible.

(2) Because of its low moisture content dried beef contains $3\frac{1}{4}$ times as much protein as raw meat, nor does it require refrigeration.

(3) Bags of dried beef have been stored for twelve months without deterioration in sacks treated with B.H.C. to prevent insect damage. Such storage must be dry, since if the product becomes wet it is spoiled by mould growth.

(4) Dried beef can be consumed in a wide variety of ways since when it is soaked it absorbs water readily and then resembles fresh meat, and may be treated as such. It is quite palatable dry and raw; it can be eaten stewed, fried or minced, or as the basis of soups.

Since no high temperatures are used in drying, it is reasonable to presume that the nicotinic acid and riboflavin remain unchanged and that a good proportion of the ascorbic acid is retained. It is clear from the analysis that there is no loss of mineral matter, the figure for ash in dried beef being $3\frac{1}{2}$ times as high as that of fresh meat.

Dried beef is being sold at Sh.1/10 cents per lb.; since it is worth, on a protein and mineral basis, $3\frac{1}{4}$ times as much as fresh meat, the equivalent price for boneless fresh meat would be 34 cents per lb., appreciably below the cheapest meat in East Africa.

### Beef Powder

Because this type of product has been found to be so valuable and so well suited to tropical conditions, an even more concentrated product made from the whole animal has been the subject of research. It has been shown that the

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whole animal excepting bone, horn, hoof and guts can be converted into a dry meat *flour*, which has been given the name of Beef Powder. This product, which is dried to 4.25 per cent moisture in special mechanical driers, is further enriched by the addition of the concentrated liquor recovered from the bone autoclave, consisting of all the water-soluble extractives as well as up to 80 per cent of the total bone protein.

To prevent rancidity the fat content is reduced to under 13 per cent by

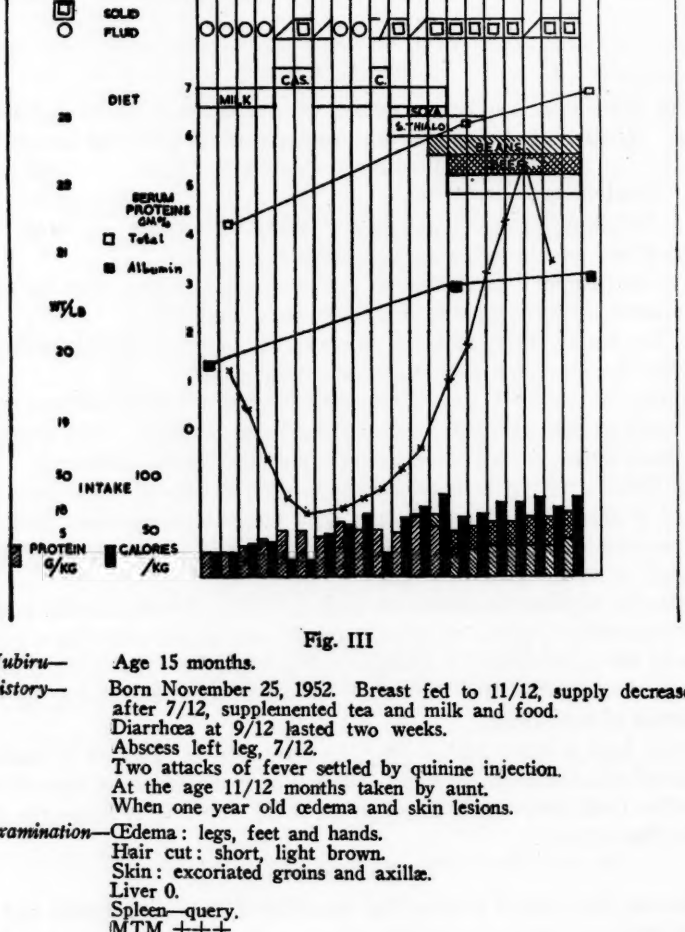


Fig. III

*Mubiru*— Age 15 months.

*History*— Born November 25, 1952. Breast fed to 11/12, supply decreased after 7/12, supplemented tea and milk and food.
Diarrhœa at 9/12 lasted two weeks.
Abscess left leg, 7/12.
Two attacks of fever settled by quinine injection.
At the age 11/12 months taken by aunt.
When one year old œdema and skin lesions.

*Examination*—Œdema: legs, feet and hands.
Hair cut: short, light brown.
Skin: excoriated groins and axillæ.
Liver 0.
Spleen—query.
MTM +++
Hookworm Neg.

*Progress*— As shown on chart.

passage through a centrifugal fat extractor. This beef powder has the following analysis:—

| | |
|---|---|
| Crude protein ... ... ... | 78.3 per cent |
| Fat ... ... ... ... | 12.7 per cent |
| Ash ... ... ... ... | 4.73 per cent |
| Water ... ... ... ... | 4.25 per cent |

Beef powder is cheap and easily stored and transported. It may be used as a protein food by itself, or used as an animal protein and mineral concentrate to supplement maize meal.

The figures generally quoted for the protein requirements of humans imply the assumption that a substantial proportion of the intake is in the form of protein from animal sources or, as it used to be called, "first-class protein." There is hardly any evidence at present that animal protein has any intrinsic superiority over vegetable protein except by virtue of the fact that its amino-acid make-up corresponds more closely with what the human organism needs; if animal protein is incorporated in the diet, therefore, these needs can be fulfilled with less over-all wastage of nutritional material. Proteins derived from vegetable staples vary among themselves in the extent of their incompleteness in respect of the so-called "essential" amino-acids; for example, cassava is an extremely poor source both in quantity and quality, while rice furnishes a protein nearly comparable with that from meat.

### Meat-enriched Maize Meal

Maize, the commonest staple with Kenya Africans, is intermediate between the two just mentioned. Its most notable amino-acid deficiency is in regard to tryptophan, and this is specially important because tryptophan is convertible in the human body to nicotinic acid, the vitamin whose shortage gives rise to pellagra. When it is remembered that maize diets are notoriously productive of this disease, both on account of the relative paucity of nicotinic acid in maize and the suspected presence of an antagonist to the vitamin in high-extraction meal, the benefit to be derived from reinforcing maize with a protein rich in tryptophan needs no emphasis.

The present legal minimum ration of fresh meat is 1½ lb. or 681 grammes per man weekly. On a protein basis this is equivalent to 136 grammes of protein. The analysis of beef powder shows a crude protein figure of 78.3 per cent; nevertheless, to allow for the non-protein nitrogen and the possibility of variation in composition, 62 per cent true protein was used for these calculations. Then on a protein basis the present minimum ration of meat would be met by 220 grammes of beef powder per week or 31.4 grammes per day. The daily maize meal ration is 1½ lb. or 681 grammes, hence an addition of 4.6 per cent beef powder to the maize meal would supply the protein in the minimum ration of fresh meat. The cost of 1 lb. of the cheapest meat is Sh.1/05, whereas the cost of 220 grammes of beef powder would be -/73 cents. Hence if the maize meal were to be fortified the labourer would receive animal protein daily rather than weekly, with nutritional advantage and there would be a saving of 30 per cent in the cost.

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The effect of adding 4.6 per cent of beef powder to 1½ lb. of maize meal would be very nearly to double the daily tryptophan intake, raising it from 0.4 grammes (barely in excess of minimal nutritional needs, without having regard to the additional requirement for nicotinic acid synthesis) to 0.75 grammes. Tryptophan is only one among eight essential (i.e., irreplaceable) amino-acids, whose daily intake would in like manner be considerably augmented.

A very important and widespread fault in most East African dietories is a gross deficiency of calcium. An accepted value for *optional* (not minimal) adult human requirement is 1,000 milligrams per day. Even if we assume, as we probably can, that this may safely be cut down by one-half, the fact remains that no more than 82 milligrams are furnished by 1½ lb. of maize meal. The incorporation of 4.6 per cent of beef powder would add a further 100 milligrams or so, a highly significant improvement, but the total would still be inadequate. However, an excellent opportunity of rectifying this is afforded by the abundant potential supply of bone meal from the same source. An incorporation of this into the fortified maize meal to the extent of 0.4 per cent, that is, one-tenth of an ounce per day, would supply a further 900 milligrams of calcium. Even allowing for the fact that, since the calcium is present as phosphate, its availability to the human organism would not be complete, the reproach of calcium deficiency could, nevertheless, no longer be levelled against the diet.

Palatability tests, involving admixture of the substance in the proportion alluded to above have been uniformly successful—the product has indeed been received, wherever tried, with acclaim.

As well as its use as a food in everyday life, beef powder has been used in the treatment of diseases derived from malnutrition such as *kwashiorkor*. The nutritional team at Mulago Hospital, Kampala, has found it to be acceptable to the patient, and palatable, while no undesirable side effects such as diarrhœa have been observed.

In view of the success of this method of destocking and the wide demand for its products, two more field abattoirs are being installed in other pastoral areas of Kenya, where disease restricts cattle movement, and "culls" are in such poor condition as to be unable to move on foot or their value does not justify transport.

**Summary**

Earlier destocking schemes aimed at drawing off fair quality slaughter animals, which could walk to the consuming areas, normally over considerable distances.

These schemes failed since the African would not sell his best stock, but offered scrub cattle only, which could not reach the market and were not of butcher's quality.

In Kenya an entirely new approach has proved itself.

The abattoir is taken to the beast, and the poorest stock drained off from the overstocked area.

The mechanics of stock purchase and the manufacture and sale of products at the Samburu Field Abattoir are described.

Special reference is made to a highly concentrated beef powder derived from the whole animal excluding only intestines and hoof and horns. This powder is designed as a protein supplement for daily maize meal rations.

Acknowledgment

The permission of the Director of Veterinary Services, Kenya, to publish this article is gratefully acknowledged.

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# THE SEQUENCE AND THE LEVELS OF THE HELMINTH INFESTATIONS IN SCOTTISH HILL SHEEP

By I. W. PARNELL

*Rugby.*

## Introduction

THE helminth infestations in Scottish hill sheep are of particular interest, because the sequence and levels of the infestations can be studied in animals which live under conditions which are less influenced by man than are those of most domestic mammals. They are of considerable economic importance. There are about 2,400,000 breeding gimmers and ewes, and probably about three-quarters of a million other sheep, excluding the 2,000,000 lamb crop, on Scottish hills. On the hills of England and Wales the sheep population is only slightly smaller. Sheep are the mainstay of hill farming. It is only on a very small proportion of hill farms that cattle are not just a side-line. Moreover, the cast Blackface and Cheviot ewes are indispensable to breed the Greyfaces and Half-breds, which are such an important factor on many marginal and low-ground farms. Some years ago it was estimated that the annual loss from worms in Scottish hill sheep was over half a million pounds sterling, and evidence gathered since then suggests that this estimate is too low. It is interesting to note that in the U.S.A. it has been estimated that losses from worm parasites amount to twice as much as losses from all other diseases put together. There is little doubt that, when better anthelmintics are available and even with the present anthelmintics, losses from worms in hill sheep could be reduced enormously by prophylactic anthelmintic dosing at the right times.

Since Morgan and Sloan (1947) first started periodic worm egg counts on Scottish hill sheep in 1944, many thousands of worm egg counts and several hundreds of post-mortem worm counts have been made to determine the sequence of the different species and the levels of the infestations in Scottish hill sheep (Morgan *et al.* 1950, 1951, 1952; Wilson *et al.* 1953; Parnell *et al.* 1954, 1955). In addition fæces have been cultured and the larvæ from them identified and counted to observe changes in the helminth populations after dosing.

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This paper is a synopsis of these seven papers; it shows the average levels of infestation at the different times of year of the species of greatest pathogenic importance in hill sheep. After a severe winter the infestations are heavier than after an average winter.

### Seasonal Variations in Worm Egg Counts

Worm egg counts were made on hill sheep of all ages at intervals ranging from a few weeks on some farms to occasional counts or a single count on other farms; these have shown the general pattern of the helminth infestations in Scottish hill sheep.

Early in June* a few eggs of "Other Strongyles"** appear in the fæces of the older lambs. On most Scottish hills the worm egg counts increase rapidly until the middle of July, when a slight fall in the number of eggs per gm. of fæces starts; this decline in worm egg counts continues until early in August. On the West Coast, however, the worm egg counts remain quite low until the beginning of August. In August another rapid increase in worm egg production occurs in all Scottish hill lambs, and the numbers continue to increase for several weeks. The worm egg counts remain high for the greater part of the autumn. In late autumn, a decrease occurs on most hills, which lasts until late winter, but the worm egg counts of hoggs never fall as low as those of older sheep, which fall to very low levels in January.

In March the worm egg counts of all ages of sheep start to rise again, and by the end of May or the beginning of June the worm egg counts are high; those of hoggs sometimes remain at high levels in the spring and early summer for longer than those of older sheep. After a severe winter the worm egg counts may be double those after a mild winter. The date of parturition affects the timing of the spring rise in gimmers and ewes. During the summer the worm egg counts fall again, rapidly at first, but sometimes in the autumn there is a tendency for them to rise slightly for a short time. This tendency occurs only in a small proportion of the ewes, and it is usually the same ewes which show this characteristic year after year.

Changes in the populations of adult helminths and other factors cause minor fluctuations in the worm egg production of individual sheep, while the major rises or falls are occurring.

### Seasonal Variations in Worm Burdens

The post-mortem worm counts were made on about seven hundred sheep which died on about eighty farms, some from each hill district in Scotland, and on about 430 slaughtered sheep from two farms, one on the Southern Uplands and the other on Loch Fyneside.

*The majority of Scottish hill lambs are born in the last half of April, some early in May and a few later in the month.

**The "Other Strongyles" include all the Strongyles found in these sheep with the exception of *Nematodirus* spp.

On most of the Scottish hills some or all of the sheep carry the following species:—*Ostertagia circumcincta, O. trifurcata, Hæmonchus contortus, Trichostrongylus axei, T. vitrinus, T. colubriformis, Cooperia curticei, Bunostomum trigonocephalum, Nematodirus filicollis, N. battus, Strongyloides papillosus, Capillaria longipes, Moniezia expansa, M. benedeni, Œsophagostomum venulosum, Chabertia ovina, Trichuris ovis, Fasciola hepatica, Dictyocaulus filaria, Muellerius capillaris* and *Cysticercus tenuicollis*. A few other species occur more rarely, such as *N. spathiger, C. onocophora, Coenuris cerebralis* and others. *Ostertagia* spp., *Trichostrongylus* spp., *B. trigonocephalum* and *C. ovina* are of pathogenic importance in large numbers of sheep on most hills. *H. contortus* is numerous in a few sheep on a few hills. *Nematodirus* spp. may be sufficiently numerous to be harmful in lambs in a few districts. Heavy infestations of *D. filaria* occur occasionally, and *F. hepatica* can be a serious cause of loss on some farms.

The levels of the infestations in Scottish hill sheep vary from year to year, from district to district and from sheep to sheep. Tables I and II, which are based on post-mortem worm counts of the slaughtered sheep and of sheep which died, show the typical levels and sequence of the infestations in an "average" Scottish hill sheep throughout her life on the hill. In the case of a few species the fact that a large number of the slaughtered sheep were from Loch Fyneside may have altered the levels of the averages to a slight extent, as on the West Coast some species are more numerous and other species are less numerous than in the rest of Scotland, but the times of the main increases and decreases of the different species are not materially affected; an asterisk (*) shows where the average has been decreased, and a dagger sign (†) where the averages has been increased. These tables are designed to show the species which are most likely to be harmful and the months of their most serious incidence, and should be the basis of any prophylactic anthelmintic dosing programmes for Scottish hill sheep. However, these averages are based on the whole month, and, therefore, for dosing at the beginning of the month the averages for the proceeding month must be considered and at the end of the month the averages for the following month must be borne in mind.

Unlike sheep on low ground, hill sheep can seldom be put on to clean ground after dosing, and therefore they are subject to reinfestation immediately after treatment. On the hill sheep of all ages graze together. Normally the only occasions when hill sheep do go on to clean ground is when hoggs are sent away to wintering, or when sheep move to the high tops in late spring or early summer in the Highlands.

### Seasonal Variations in the Numbers of Larvæ

Crofton (1949) has shown that the numbers of infective larvæ on hill pastures in Northumberland start to increase rapidly during May or June, and reach their peak towards the end of August, then the numbers decrease again until by the end of October the numbers on the grass are comparatively small.

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## TABLE I.

*A table showing the average infestations of a "typical" Scottish hill sheep during her life as a lamb and as a hogg.*

| | Lamb | | | Hogg | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May | June |
| *Ostertagia* spp. ... ... | 70 | 640 | 720 | 1800* | 880* | 460 | 380 | 400* | 860* | 2900* | 3600* | 2700* | 1200 |
| *H. contortus* ... ... | 2 | 2 | 15† | 60 | 190 | 18 | 16† | 3 | 9 | 8 | 120 | 1040† | 360† |
| *T. axei* ... ... ... | 0 | 16 | 60‡ | 520* | 100* | 130* | 480* | 420* | 680* | 1300* | 1400*‡ | 340* | 1100‡ |
| Larvæ in abomasum ... | 3 | 40 | 140 | 80 | 50 | 30 | 480 | 200 | 1700 | 260 | 1700* | 300 | 60 |
| *Trichostrongylus* spp. in small intestine ... | 15 | 60 | 200 | 520 | 860* | 480 | 740 | 700 | 720 | 4500* | 3000 | 960 | 660 |
| *B. trigonocephalum* ... | <1 | <1 | 12 | 14 | 20 | 50 | 50† | 50† | 120† | 60 | 60 | 80† | 100† |
| *Cooperia* spp. ... ... | 3 | 5 | 40 | 120 | 90 | 40 | 50 | 120‡ | 50 | 60 | 180‡ | 20 | 220‡ |
| *Nematodirus* spp. ... | 80 | 140 | 200 | 320 | 420 | 380 | 740 | 560 | 300 | 300 | 960 | 150 | 120 |
| Larvæ in small intestine | 10 | 20 | 5 | 25 | 50 | 20 | 40 | 30 | 20 | 40 | 200 | 80 | 15 |
| *C. ovina* ... ... ... | <1 | 2 | 1 | 3 | 3 | 2 | 7 | 4 | 18 | 60* | 50* | 20† | 4 |
| *Œ. venulosum* ... ... | <1 | <1 | 5 | 9 | 6 | 7 | 8 | 8 | 20 | 70 | 100 | 80† | 34† |

* Average counts lowered by the counts from the slaughtered sheep from a West Coast farm.
† Average counts raised by the counts from the slaughtered sheep from a West Coast farm.
‡ Average counts raised by the counts from the slaughtered sheep from an Ettrick farm.

## TABLE II.

*A table showing the average infestations of a "typical" Scottish hill sheep during her life as a gimmer or ewe.*

| | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May | June |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ostertagia* spp. ... ... ... | 1700 | 400 | 1600* | 2400* | 900* | 400 | 250 | 850* | 2000* | 2200* | 3700* | 2900* |
| *H. contortus* ... ... ... | 90† | 25 | 70† | 20 | 30 | 5 | 1 | 0 | 4 | 40 | 780 | 380* |
| *T. axei* ... ... ... ... | 1000* | 420‡ | 240 | 400* | 260* | 180* | 300 | 340* | 860* | 440 | 800‡ | 1000* |
| Larvæ in abomasum ... ... | 150 | 100 | 80 | 540 | 200 | 90 | 300 | 100 | 900‡ | 1900‡ | 800† | 150 |
| *Trichostrongylus* spp. in small intestine ... ... ... | 1100 | 280 | 1100 | 2900* | 720 | 100 | 140 | 540 | 1400 | 640 | 2500* | 1200 |
| *B. trigonocephalum* ... ... | 22 | 22 | 11 | 18 | 14 | 18 | 34 | 16 | 28 | 30 | 14 | 24 |
| *Cooperia* spp. ... ... ... | 300‡ | 320 | 120 | 480 | 200 | 40 | 140 | 260 | 160 | 300‡ | 50 | 300‡ |
| *Nematodirus* spp. ... ... | 240 | 20 | 180 | 30 | 100 | 5 | 50 | 10 | 180 | 120 | 120 | 200 |
| Larvæ in small intestine ... | 1 | 20 | 30 | 320* | 740* | 5 | 20 | 40 | 50 | 60 | 40 | 60 |
| *C. ovina* ... ... ... ... | 24 | 10 | 9 | 10 | 4 | 5 | 5 | 7 | 48* | 36* | 34 | 40 |
| *Œ. venulosum* ... ... ... | 6 | 9 | 5 | 12 | 2 | 2 | 4 | 4 | 30* | 5 | 14 | 10 |

* Average counts lowered by the counts from the slaughtered sheep from a West Coast farm.
† Average counts raised by the counts from the slaughtered sheep from a West Coast farm.
‡ Average counts raised by the counts from the slaughtered sheep from an Ettrick farm.

The seasonal variations in the numbers of larvæ in hill sheep is very different, presumably because of the effect of resistance on their fate after ingestion. Although a few larvæ were found in the abomasa of lambs early in June, the numbers were still low in July. Subsequently the numbers from the different sources varied, but were comparatively low until the end of the year. Quite early in the year the numbers increased, and in hoggs in late winter and early spring large numbers were found. It is probable that the average of 260 (see Table I) found in hoggs in March would be higher if the average had been based on a larger number than 26. Only a very few larvæ were found embedded in the mucosa of the abomasa. The greatest number was 121, which suggests that under Scottish hill conditions only a few lie dormant, although Sommerville (1953-1954) found that when large numbers of larvæ of *O. circumcincta* were given to sheep many remained dormant in the mucosa of the abomasum. During the late spring and summer the numbers of immature stages found in hoggs were quite low again. In mid-autumn there was a tendency for the numbers of larvæ to increase slightly in gimmers and ewes. In March, April and May, particularly in April, the numbers of larvæ in the abomasa of adult sheep was very high, although throughout the year very few were found in the mucosa of gimmers and ewes.

The numbers of larvæ in the small intestines were usually considerably lower than in the abomasa; occasionally, however, large numbers were found, sometimes when there were only a few in the abomasum; some of these larvæ may have been larvæ which were being expelled from the abomasum. The October and November averages for ewes may be an example of this. The proportion of larvæ which become established depends largely on the age and condition of the sheep. Generally the helminth infestations of gimmers are higher than those of ewes. Table II was prepared by averaging gimmers and ewes together, as individual differences between sheep are considerably greater, and an "average" table of this nature can only be a general guide.

### The Adult Helminth Infestations

*Ostertagia spp.*

On most Scottish hills *Ostertagia* spp. are the first species of pathogenic importance to become numerous in lambs. Quite early in June some reach maturity; by July there may be several hundreds in lambs in south-east Scotland, and by August the average everywhere is several hundreds. Until the end of July over half of the Other Strongyle eggs in lamb fæces are those of *Ostertagia* spp. The numbers of this genus tend to increase in lambs until early autumn, when there is a fall in numbers until after the New Year. Worm egg and larval counts suggest, however, that Table I shows a more rapid increase in late summer than is typical. In February and in March the numbers increase in hoggs until there may be some thousands at the end of March, in April and in May. After three or four months of high counts the numbers fall considerably. In late summer and early autumn the numbers of *Ostertagia* spp. in gimmers and in ewes tend to increase again slightly for a short time, but by the end of

the year the infestations are very light. In February and March the infestations in breeding sheep build up considerably, and during the following three or four months infestations of a few thousands are normal, and of several thousands quite common.

Infestations of *Ostertagia* spp. tend to be later and less heavy in hill sheep near the West Coast than in hill sheep in the south of Scotland.

*O. circumcincta* outnumbers *O. trifurcata* by about 4 to 1, the difference in the ratio is rather greater north of the Forth, especially on the West Coast.

In Scotland in cases of " black scours " there are usually several thousands of *Ostertagia* spp. and of *Trichostrongylus* spp. If the outbreaks are not checked immediately the death rate in hoggs may exceed 20 per cent, and the survivors seldom make good stock sheep.

*Hæmonchus contortus.*

*H. contortus* is not of much pathogenic importance to most hill sheep in Scotland. Many lambs harbour a few, but even on those farms where it is comparatively numerous, it is not until late summer or early autumn that the infestations build up. During winter the infestations in hoggs are very light, and do not increase again until April or May. In gimmers and in ewes the winter infestations are also very light, and it is usually late spring before heavy infestations occur, and they usually continue only for a comparatively short time, although there may be some throughout the summer. Although very occasionally heavy infestations occur all over Scotland, they appear to be most common on the hills running east from the Moor of Rannoch to Aberdeenshire, on the West Coast and on the hills south of the Firth of Forth, where there are areas of heather clad hills with patches of short grass. Since the free living stages of *H. contortus* are unlikely to survive the winter on most Scottish hills, especially where night frosts are common after warm days, removal of the few adults from a whole flock in autumn and in winter should result in its virtual extinction in some areas. The prolific egg production of this species is one cause of high worm egg counts in late spring and summer.

*Trichostrongylus axei.*

Only a very few *T. axei* are found in Scottish hill lambs before the end of August; subsequently the numbers increase. During the autumn infestations usually number a few hundreds. *T. axei* is one of the earliest species to increase in numbers during the winter in hoggs and in older sheep. This increase in numbers persists for three or four months. Throughout the rest of the year gimmers and ewes normally harbour a few hundred *T. axei.*

*Trichostrongylus vitrinus and T. colubriformis.*

The pattern of the infestations of the *Trichostrongylus* spp. of the small intestine is very similar to that of *T. axei,* but the infestations are generally heavier. The average ratio is approximately one *T. axei* to three *T. vitrinus* and *T. colubriformis,* except north of the Forth where the ratio averages 1 to 2. In most districts *T. vitrinus* outnumbers *T. colubriformis* by about 5 to 1. These two species are of considerable pathogenic importance in hoggs because their

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numbers tend to be maintained throughout the winter. There is an increase in numbers in the autumn in some adult sheep.

*Bunostomum trigonocephalum.*

Infestations of *B. trigonocephalum* build up fairly slowly in lambs and it is not until autumn that the numbers are sufficient to be of pathogenic significance, even allowing for the suggestion that this hookworm is the most pathogenic of all the nematodes found in Scottish hill sheep. The numbers vary considerably from sheep to sheep, but on the average a fairly constant level of infestation is maintained in adult sheep throughout the year, and there is no marked fall in numbers at the end of the year nor is there a spring increase, comparable to that of many other species. On the West Coast the infestations tend to be heavier than in most other districts.

*Nematodirus spp.*

The serious outbreaks of nematodirosis which occur in Cross lambs in south-east Scotland have not been reported in hill lambs. However, some of the infestations of *Nematodirus* spp. in late June and in July, judging by egg counts, in lambs on hills in the basin of the Border Esk, and occasionally elsewhere, may be sufficiently heavy to do some damage. Later the infestations of species of this genus build up in hoggs until the spring, when the numbers decrease rapidly. In older sheep the infestations are always light.

*Cooperia spp.*

The infestations of *Cooperia* spp. are very light in lambs and in hoggs. In older sheep there are often a few hundred adults. The heaviest infestations occur in summer. Some of the average counts in Tables I and II have been increased by the *Cooperia* spp. which were in the slaughtered sheep from the Ettrick Valley; this is shown by a double dagger sign (‡) in the tables.

*Chabertia ovina.*

It is not until the hoggs are nearly a year old that *C. ovina* becomes sufficiently numerous to be of pathogenic importance; this spring rise in numbers lasts for some weeks, and occurs at the same time of year in gimmers and in ewes, but perhaps lasts longer in them.

*Œsophagostomum venulosum.*

In hoggs in autumn and in spring the average counts of *Œ. venulosum* are greater than those of *C. ovina,* but in older sheep *C. ovina* outnumbers *Œ. venulosum,* although its numbers do increase slightly in the spring.

*Trichuris ovis.*

*T. ovis* is one of the earliest species to reach maturity in Scottish hill lambs, but it does not become sufficiently numerous to be harmful. Sheep over about a year old harbour only very small numbers.

*Fasciola hepatica.*

There are few Scottish hill farms which are completely free of fluke, but the proportion of sheep which are infested differs considerably from district to district. On some farms, particularly in the west, there may not be an adult

sheep which is free from it, but on other farms only a very few sheep may be infested. Not until early autumn does *F. hepatica* reach maturity in Scottish hill lambs and from then on the infestations can build up. The potential life of *F. hepatica* has been shown by Durbin (1952) to be longer than that of most hill sheep, which are usually sold for breeding Greyface or Halfbred lambs on marginal or low ground farms when they are $5\frac{1}{2}$ or $6\frac{1}{2}$ years old; therefore, unless anthelmintic treatments remove them, the incidence of fluke infestations tend to increase with the age of the sheep.

**Summary**

In most of Scotland *Ostertagia* spp., and in some districts *Nematodirus* spp., are the first species of pathogenic importance to infest hill lambs in sufficient numbers to do damage.

In late summer and early autumn infestations of *Trichostrongylus* spp., of *B. trigonocephalum* and, in some areas in a few lambs, of *H. contortus* also become sufficiently heavy to damage lambs.

In late winter and early spring *Ostertagia* spp., *Trichostrongylus* spp., *C. ovina* and *O. venulosum*, and later *H. contortus*, may be very numerous in hoggs, and infestations of *B. trigonocephalum* may have persisted throughout the winter.

*B. trigonocephalum* also persists in adult sheep throughout the year.

In breeding sheep *Ostertagia* spp. and *Trichostrongylus* spp. of the small intestine may be numerous in the late summer and autumn. The very occasional heavy infestation of *Cooperia* spp. also occurs in summer.

In gimmers and ewes infestations of *Ostertagia* spp., of *Trichostrongylus* spp. and of *C. ovina* increase in late winter and early spring, followed by some heavy infestations of *H. contortus* in a few areas.

The incidence of fluke varies considerably from district to district and even from heft to heft; this parasite may live for several years in sheep.

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# STUDIES IN BOVINE MALIGNANT CATARRH

## V. The Role of Sheep in the Transmission of the Disease

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THE part played by sheep in the transmission of bovine malignant catarrh has long exercised the minds of workers investigating this disease. A belief that sheep act as a virus reservoir has been held by scientists in Europe since the turn of the century, and it was in 1930 that Götze produced experimental evidence in support of this view. Sheep were removed from an infected farm and placed in contact with healthy susceptible cattle. Sixteen of fifty animals exposed died of malignant catarrh.

This concept was supported by field observations of outbreaks amongst cattle in similar circumstances; there was, however, a conflict of opinion as to the significance of sheep. For example, at the 13th International Veterinary Congress, held in Switzerland in 1938, Rinjard said that sheep harbour the disease, and that the virus is transmitted in occult form from generation to generation, and that infection can remain in sheep for several years without reinfection from cattle. Wysmann, whilst admitting that the association of sheep and cattle may contribute to the spread of the disease, stressed that outbreaks also occur in the absence of sheep, and that other unknown carriers must be implicated. Du Toit and Alexander, on the other hand, pointed out that in South Africa the wildebeeste is the principal carrier, and considered that the view held in Europe that a connection exists between sheep and the incidence of malignant catarrh in cattle should not be accepted without further confirmation. In the discussion that followed the presentation of these papers, Daubney stated that it had not been possible to observe any connection between sheep and cattle in the transmission of malignant catarrh in East Africa.

Thus, until recent years, workers in Europe were strongly of the opinion that sheep play an important part in the transmission of malignant catarrh, whilst African authorities inclined to attach little significance to this view. In 1950, however, de Kock and Neitz in South Africa repeated Götze's (1930) work and infected cattle by placing them in close contact with sheep obtained from an infected farm. Of eight susceptible animals exposed in this way, two died of malignant catarrh after incubation periods of nine and ten weeks respectively. The same sheep were again confined with cattle after an interval of ten and a half months, and no further cases occurred. This suggests that the sheep had, in the interim, lost their infection and is in direct contrast to the findings of Rinjard (1938) who claimed that the virus could remain in a flock of sheep for over four years without reinfection from cattle. De Kock and Neitz conclude by stating, " in certain areas of the Union, sporadic cases

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are from time to time met with where the reservoir host is undoubtedly the sheep."

### 1.—Field Observations

In Kenya, despite Daubney's (1939) observation that "further work on this disease has failed to demonstrate any connection between sheep and the infection in bovines," a firm opinion is held amongst many field officers that credence must be given to the continental view. The outbreak at the Ngong African Veterinary Training Centre, to which reference has been made in an earlier article (Piercy, 1952*a*), provides important circumstancial evidence in support of this view.

Malignant catarrh had been endemic amongst Zebu stock at the Ngong farm for many years. Two groups of cattle are maintained, a herd of dairy cows and young female stock based on the home farm, and a ranched herd which is kept on another part of the farm some distance away. Nearly all cases of malignant catarrh have occurred in the former, the ranched herd having a low incidence. It had been the practice for some years to confine the weaner herd at night in an enclosure adjacent to a flock of some 200 head of Red Masai sheep. The cattle and sheep were separated by a corrugated iron fence only. Deaths from malignant catarrh had reached serious proportions by August, 1950. In the two years 1947 and 1948, the total number of deaths from this disease was eleven. In 1949 the figure jumped to thirty-eight and during the first seven months of 1950 thirty-four more fatal cases occurred (Table I). The financial loss, together with the interference to the breeding programme, became so serious that it was decided to remove the sheep, on the assumption that some of them might be acting as virus carriers. At the same time it was agreed that all the cattle should receive an experimental formolised vaccine prepared from another strain of virus maintained at the Kabete laboratories (Piercy, 1952*a*).

TABLE I

*Incidence of Bovine Malignant Catarrh in the Ngong dairy herd*

| Year | Jan. | Feb. | Mar. | Apr. | May | Jun. | Jul. | Aug. | Sep. | Oct. | Nov. | Dec. | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1947 ... | 0 | 0 | 0 | 1 | 0 | 4 | 2 | 0 | 0 | 0 | 1 | 0 | 8 |
| 1948 ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 3 |
| 1949 ... | 1 | 1 | 1 | 2 | 6 | 2 | 5 | 7 | 1 | 3 | 2 | 7 | 38 |
| 1950 ... | 1 | 0 | 5 | 4 | 7 | 10 | 7* | 3† | 1 | 1 | 0 | 0 | 39 |
| 1951 ... | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1952 ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* = Sheep removed; † = herd vaccinated.

The sheep were removed in July, 1950, some being sent for slaughter and others to six widely separated districts. The cattle were vaccinated in August. From Table I (1950) it will be seen that three cases of malignant catarrh occurred in August, one in September and one in October, as compared with ten in June and seven in July. After this, no further cases were noted in the

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dairy herd for an unbroken period of eight months until July, 1951, when one animal died. Thereafter, no fresh cases were seen up to the end of 1952. Thus, during the two-year period 1951/2, only one case was diagnosed compared with thirty-nine in 1950, and thirty-eight in 1949.

In retrospect, it is unfortunate that the gravity of the situation necessitated the combination of two distinct measures of control and it may never be clear whether vaccination, or the removal of the sheep, or a combination of the two were responsible for the very marked reduction in cases of malignant catarrh. The value of vaccination has been discussed in a previous article (Piercy, 1954), and the conclusion was reached that although vaccinated animals may be unable to withstand artificial challenge inoculations under laboratory conditions, sufficient protection may be provided to prevent natural infection. Whatever may be the correct interpretation, however, the dispersal of the Ngong sheep was followed by a peculiar sequence of events on some of the farms to which they were sent.

The African Veterinary Training Centre at Maseno, situated in the Nyanza district of Lake Victoria, about one hundred and sixty miles away from Ngong, received nineteen of the suspect sheep. A herd of pure-bred Zebu cattle is maintained at this station and malignant catarrh was unknown before the arrival of the sheep. No sheep had been kept at this station for many years. The young weaner herd, fifty-eight in number, was housed at night in an open-eaved building some 20 feet away from the night enclosure of the sheep. In May, 1951, some ten months after the arrival of the sheep, a yearling steer contracted malignant catarrh and died. Two more yearlings died within two weeks and three further fatal cases occurred during the next five months. The diagnosis of malignant catarrh on clinical grounds was made by the Provincial Veterinary Officer (Lewis, 1951) who had been stationed at Ngong and was thoroughly conversant with the disease. Post-mortem and histological examinations confirmed the diagnosis.

All these deaths took place amongst the weaner herd. Other cattle on the farm with which the sheep had no kind of contact were unaffected.

In February, 1951, the sheep were transferred to an adjoining Training Centre at Siriba, situated about a mile and a half away. They were housed at night in a grass hut within 100 yards of the night quarters of a herd of Zebu dairy cattle of mixed ages. During the day the sheep grazed over the same pasture as the younger cattle. In October, 1951, eight months after the arrival of sheep at Siriba, four fatal cases of malignant catarrh occurred amongst the cattle; the ages of the affected animals were six months, one and a half years (2) and eight years respectively and the disease lasted for two, three, six and fourteen days respectively. There was no difficulty in confirming the diagnosis by the histological examination of affected tissues. Three more cases of malignant catarrh, all in young heifers, occurred during the first week of December.

In November, 1951, the sheep were transferred to Kabete and placed in a four-acre paddock with eight cattle for twelve months. Fresh cases of malignant

catarrh at Maseno ceased within four months, and at Siriba within one month of the removal of the sheep. At Kabete, the contact cattle remained healthy.

Another group of thirty Ngong sheep was sent to Mr. " E's " farm at Athi River, about thirty miles east of Ngong. The sheep had contact with a herd of native Boran cattle and were housed in adjacent "bomas" separated by a thorn fence. In October, 1951, three months after their arrival, three deaths from malignant catarrh occurred amongst young heifers. The sheep were removed to another part of the farm and the cattle received a course of vaccine. Thereafter, no further cases of malignant catarrh were diagnosed.

From the other four farms to which Ngong sheep, in small groups of 12-20 were sent, no reports of the appearance of malignant catarrh have been received, although from one of them (Mr. " H's " farm at Turbo in the Kenya Highlands), comes an account of an unidentified disease simulating East Coast fever.

Further interesting evidence in favour of the significance of sheep in the presence of bovine malignant catarrh comes from an outbreak of the disease which was diagnosed in 1951 amongst cattle on a farm in the Kitale area of the Trans-Nzoia district, situated in the northern part of the Kenya Highlands. The outbreak was diagnosed in two herds of native Boran and high-grade Ayrshire cattle. In June, July and August, 1951, losses were heavy and during one period of six weeks fifty deaths occurred, mostly in grade stock. There was contact, during grazing, between these cattle and a flock of native and low-grade Karikul sheep which had been brought on to the farm in January, 1950. The farm from which these sheep came is in the Hoey's Bridge district, some 25 miles away, where malignant catarrh, so far as is known, has never been diagnosed.

Acting on the advice of the District Veterinary Officer (Holmes, 1951), the sheep were removed early in July, 1951. No other steps were taken to control the outbreak apart from the isolation of sick animals and the hygienic disposal of carcases. By the end of September, two and a half months after the removal of the sheep, fresh cases of malignant catarrh had ceased to occur.

### 2.—Laboratory Experiments

*Materials and Methods.*

*Virus strain.* The " D " strain of bovine malignant catarrh virus (Piercy, 1952*a*), maintained in cattle at Kabete by serial passage.

*Inoculations.* Unless otherwise stated all cattle were inoculated into the prescapular gland. Inocula, such as gland and spleen, were prepared by the maceration of 20 per cent of the material concerned in sterile sand and normal saline. Suspensions were not centrifuged since it is known that malignant catarrh virus is rapidly deposited by this means. All inoculations were made on the same day as the material was harvested.

*O.C.G. preservative.* This consisted of a mixture of 500 parts glycerine, 500 parts distilled water, 5 parts phenol and 5 parts potassium oxalate.

*Challenges.* These were made by the intraglandular inoculation of 2.0 c.c. of a 20 per cent suspension of known infective gland.

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### Attempts to Infect Sheep from Cattle

On August 11, 1950, three cross-bred Merino wethers were inoculated with gland taken from a bovine animal (9629) which was reacting to experimental infection. Sheep 9023 and 8983 each received 5.0 c.c. intravenously, and sheep 156 2.0 c.c. into the tissues surrounding the left prescapular gland. All three sheep were immune to Bluetongue and two of them (8983 and 9023) to Nairobi sheep disease. Between six and seven weeks later, they all contracted an illness characterised by general debility, weakness of the hind limbs, a stilted gait, enlarged superficial lymph glands, and a slight, mucous nasal discharge. Sheep 9023, which was febrile for three days, reaching a peak of 107° C., became weaker and died on October 16, 1950, just over two months after inoculation. The other two sheep were destroyed about the same time when in a weak state. Post-mortem examinations revealed a number of lesions common to all three, the most striking of which was a general enlargement, with some œdema, of the lymph glands. There was also some evidence of an encephalitis. In one case a leucocytic infiltration of the liver was present and in another similar changes were seen in the kidneys. Two of the spleens had dark, soft pulps, and hyperplasia of the malphigian corpuscles was seen in one of them. Histological and biological examinations for heartwater were negative. Apart from a fairly heavy infestation of *O. columbianum* in sheep 156, intestinal parasites were not seen. Histo-pathological changes characteristic of malignant catarrh in cattle were not found in sections of liver and lymph gland.

Subinoculations to five other sheep were carried out as follows: 2.5 c.c. of lymph gland from sheep 8983 was given intravenously to sheep 199 and 251, and by intraglandular inoculation to a bovine animal 343; 10.0 c.c. of blood in O.C.G. preservative, 1.0 c.c. of lymph gland and 1.0 c.c. of spleen from sheep 156 were given intravenously to sheep 105, 117 and 111 respectively. In addition, 1.5 c.c. of spleen and gland from sheep 156 were given to bovine animals 8410 and 9600.

None of the cattle reacted and they were later shown to be susceptible to challenge. Of the sheep, the only one to show clinical evidence of ill-health over a six months period of observation was number 251 receiving lymph gland intravenously which experienced a slight and irregular temperature reaction between the twenty-sixth and thirty-first days after inoculation, followed by a mild nasal catarrh and a noticeable enlargement of the left prescapular lymph gland. This animal then returned to normal health and was sacrificed for examination on February 15, 1951, some five months after inoculation. The post-mortem examination revealed no macroscopic lesions and portions of liver, kidney, spleen and lymph gland, were normal by histological examination.

To investigate the possibility that sheep 251 might be harbouring virus after recovery from a mild attack of malignant catarrh, portions of liver, lymph gland, kidney and spleen were pooled and given to a bovine animal 770. This animal did not react. When challenged two months later, however, it failed to react for the second time. After a further interval of three months it was challenged again, whereupon it reacted and died of malignant catarrh. Since

natural resistance to laboratory challenge with the "D" strain of malignant catarrh virus is extremely rare (Piercy, 1952*a*), and since it is known from the results of other experiments (Piercy, 1954) that an artificially induced immunity disappears within a few months, it is conceivable that the inoculation of this animal from sheep 251 was responsible for a temporary immunity.

### Attempts to Infect Cattle from Suspect Carrier Sheep

(i) *By inoculation.* On May 27, 1951, a sheep which had been housed for eight weeks in the same stall as a succession of cattle reacting to malignant catarrh, was destroyed. During this time the sheep had remained healthy. A 10 per cent suspension of equal portions of liver, spleen and lymph gland were prepared. Of this mixture 10.0 c.c. was given to a bovine animal 1767. No reaction occurred over a six months period of observation.

Earlier in this article an account has been given of malignant catarrh at the Ngong, Maseno and Siriba African Veterinary Training Centres. Six of the sheep which originally had been at Ngong and were moved to Maseno, were brought to Kabete on July 14, 1951. This was one year after they had been in contact with malignant catarrh at Ngong and two months after contact with the disease at Maseno. One of these sheep, selected at random, was destroyed and 50.0 c.c. of citrated blood given intravenously to bovine animal 1951, whilst number 1955 received 7.0 c.c. of a suspension of equal parts of spleen, lymph gland and liver by intraglandular inoculation. Neither of these animals reacted and when challenged three and a half months later they were shown to be susceptible.

(ii) *By contact.* On February 22, 1951, three cross-bred Merino wethers, obtained from an area in which malignant catarrh is unknown, were placed in the same stall as two steers reacting to experimentally induced malignant catarrh. When the latter died, other reacting animals were substituted so that for a period of eight weeks, the sheep were in continuous contact with cattle in the clinical stages of malignant catarrh. During this period, the sheep remained healthy. They were then removed and placed in another stall in company with two susceptible cattle, one of which died suddenly from causes unknown twenty-six days later. The other, 1630, remained healthy for four months when it was challenged. It reacted and died of malignant catarrh. One of the sheep (see experiment (i) above) was examined for the presence of virus with negative results.

Six Ngong sheep, obtained from Maseno (see experiment (ii) above) were obtained and placed in the same stall as four susceptible steers. Two of the latter died from misadventure; the other two, 1905 and 1779, which were left in intimate contact with the sheep for ten months, remained healthy.

### Discussion

When Mettam (1923) infected cattle with the blood of an apparently healthy wildebeeste, he proved conclusively that the virus of bovine malignant catarrh can exist for unknown periods of time outside its bovine host. The work of Götze (1930), Rinjard (1938) and de Kock and Neitz, (1950) provides satis-

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factory evidence that sheep also may play an important part in the transmission of this disease. The outbreak of malignant catarrh which followed the successive movements of the Ngong sheep provide further confirmation.

These sheep had been in continuous contact with malignant catarrh for several years. It is reasonable to suppose, therefore, that a number of them were virus carriers. They were moved to Maseno and fresh cases at Ngong ceased to occur within four months. At Maseno, where malignant catarrh was previously unknown, the sheep were placed in contact with the weaner herd. Some ten months later the disease appeared in these cattle, but not in other cattle with which the sheep had no contact. When the sheep were moved to Siriba, fresh cases at Maseno ceased to occur within four months. At Siriba, cattle with which the sheep were placed in contact began to die from malignant catarrh eight months later. The sheep were then moved to Kabete and the disease disappeared at Siriba one month later.

At Kabete, however, although these sheep were in contact with cattle for 12 months, malignant catarrh did not appear. Similarly, when six of the Ngong sheep, transferred from Maseno when the disease first broke out there, were placed in contact with other cattle at Kabete, transmission was not effected after a period of ten months. Other sheep at Kabete, in continuous contact with reacting cattle for a period of eight weeks, also failed to transmit the disease to susceptible cattle.

Direct transmission of malignant catarrh between cattle by contact seldom occurs. It is commonly believed that an intermediate vector, such as a blood sucking insect, is responsible. The failure of the laboratory contact experiments, therefore, might be explained by the absence of such a vector at Kabete. Another possibility is that lodgement of virus only occurs in a small number of sheep. The number of sheep used in the Kabete experiments was small and it might well be that a larger number must be used to ensure transmission.

That malignant catarrh can occur in the absence of both sheep and wildebeeste is well substantiated and it may be noted that a single case of the disease occurred in the dairy herd at Ngong in July, 1951, after a disease-free interval of nine months. During this time the cattle had no contact with either sheep or wildebeeste. But although 1951 was marked by exceptionally heavy rains, following which the incidence of malignant catarrh commonly rises, no further case had occurred in the dairy herd by the end of 1952.

It will be recalled that the Ngong cattle were vaccinated in August, 1950, soon after the sheep were removed. The absence of fresh cases thereafter, from October, 1950 until July, 1951, when one case occurred, and again from July, 1951 to the end of 1952, strongly suggests that either the vaccination or the removal of the sheep was responsible. The laboratory vaccination trials (Piercy, 1954) suggest that the vaccine used would only protect for a few months and it seems more likely that the continued absence of the disease must be attributed to the removal of the sheep.

Attempts to transmit malignant catarrh from suspect carrier sheep to cattle by blood and tissue inoculations met with negative results. The reason for

failure may again have been that although the sheep used had been in contact with the disease, lodgement of virus had not taken place in any one of them. If the percentage of exposed sheep that become virus carriers is low, it is likely that to prove the existence of virus carriers by inoculation, a large number of sheep from a suspect flock must be used.

Failure to infect sheep with bovine material has been reported by other workers (Mettam, 1923, de Kock and Neitz, 1950). In the present experiments there was some evidence of success. All three sheep of the first generation reacted to an illness characterised by enlarged superficial lymph glands and a mild nasal catarrh, six to seven weeks after inoculation. It was possible to rule out the common East African sheep diseases, such as bluetongue, Nairobi sheep disease and heartwater. But when material from these sheep was given to three cattle and five other sheep, the former remained healthy, and only one of the latter appeared to go through a mild reaction a month after inoculation. When material from this one sheep was returned to a steer, no reaction followed but the animal was resistant to challenge two months later. After a further interval of three months this animal reacted to challenge and died.

These results are capable of two interpretations. On the one hand, the disease which affected four of the eight sheep may have been non-specific and coincidental. In this case it must be assumed that the steer that resisted challenge after inoculation with material from one of these sheep happened to possess a transient immunity which disappeared within the next three months. On the other hand, the malignant catarrh virus may have established itself in the first generation of sheep, survived in one out of five sheep in the second generation, and when returned to a steer have stimulated a temporary immunity sufficient to resist challenge two months later.

The observations recorded in this paper permit two generalisations. Firstly, the evidence from the field strongly supports the belief that sheep from a farm on which malignant catarrh is endemic, when transferred to a clean farm, may be responsible for fresh outbreaks of the disease. Secondly, the laboratory results emphasise the difficulty of proving this by artificial means.

If sheep do harbour the virus, and if transmission is affected simply through the agency of an intermediate blood sucking insect vector, there should be no difficulty in transmitting the disease from sheep to cattle by the inoculation of small quantities of sheep blood. Since success by this means has so far not been attained, it would seem either that the percentage of sheep in any contact flock that carry virus must be very low, or that the virus must undergo some essential change in the vector before it can infect cattle, or that sheep do not actually harbour the virus at all. In this connection, it may be that sheep play their part merely by proving attractive to the vector, which transmits the disease either directly from ox to ox, or from some other animal or rodent reservoirs at present unidentified.

## Summary

1. The history of a number of outbreaks of bovine malignant catarrh, believed to have been associated with the presence of sheep is described.

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2. On two farms, situated in a district where malignant catarrh was previously unknown, the disease occurred ten and eight months respectively after the introduction of sheep from an infected farm.

3. On four different farms fresh cases of malignant catarrh ceased to occur for four, one, and two and a half months respectively after the removal of sheep.

4. At Ngong, where malignant catarrh had been enzootic for many years, the removal of sheep combined with the use of an experimental vaccine was followed by a reduction of fresh cases in the dairy herd from thirty-nine in 1950 to one in 1951, and none in 1952.

5. Cattle confined in stalls with sheep from an infected farm, and with sheep which had been in continuous contact for eight weeks with animals reacting to malignant catarrh at Kabete, remained healthy for periods up to ten months.

6. Cattle inoculated with blood and tissue suspensions taken from sheep which had been in prolonged contact with malignant catarrh remained healthy.

7. The inoculation of sheep with infective bovine material was followed by an unidentified illness in a number of cases, but attempts to return the virus to oxen from such cases failed, although one steer appeared to gain a transient immunity.

8. The part played by sheep in the transmission of bovine malignant catarrh is discussed.

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# THE GROWTH OF THE CONTAGIOUS BOVINE PLEUROPNEUMONIA ORGANISM IN EGG AND ITS CONSTITUENTS

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SHERIFF AND PIERCY (1953) claimed to have attenuated the pleuropneumonia organism by passaging it through fertile eggs and to have immunised cattle with such organisms. Dafaalla and White (1953) criticised this work and recent attempts by two of my colleagues (Dafaalla and Nasri—See Annual Report 1954)—who, however, used a different technique to confirm the results, have failed.

With some idea of clarifying the position it was decided to see if the pleuropneumonia organism would grow in whole egg and its constituents.

## Technique

Cleaned eggs were wiped with cotton wool soaked in methylated spirits and allowed to dry. They were then broken, the fluid collected in a sterile enamel jug and beaten up with a glass rod until a reasonable consistency had been achieved. (Later an electrically driven Paladin mixer was used.) The fluid was collected in 9 ml. volumes and enough penicillin solution added to give a concentration of 200 units per ml. (usually 0.2 ml. of a solution of 200,000 units in 20 ml. plain broth).

Serum broth cultures were diluted to 1 in $10^4$ (to remove any effect of added serum) and 1 ml. added to the egg fluid.

Counts were made immediately and after incubation for different lengths of time.

Growth of the organism was always confirmed microscopically, using the modified stevenel blue technique and tubes showing no growth were incubated for 10 days before being discarded.

## Results

(a) *Growth in whole (fresh, infertile) egg.* Somewhat surprisingly it was noted that whole egg not only failed to support growth, but was bactericidal.

Table I shows a typical result, from which it may be seen that the count dropped from 1 in $10^5$ to less than 1 in $10^2$ in under four days. (The 1 in 10 dilutions of whole egg were always too thick to determine growth microscopically.)

TABLE I

*Growth in whole (infertile) egg*

| Medium tested | Days seeded medium incubated | Seeded medium diluted 1 in: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $10^2$ | $10^3$ | $10^4$ | $10^5$ | $10^6$ | $10^7$ | $10^8$ | $10^9$ |
| Whole egg ... ... ... | 0 | + | + | + | + | — | — | — | — |
| | 2 | + | + | + | — | — | — | — | — |
| | 4 | — | — | — | — | — | — | — | — |
| Whole egg + 10% serum | 0 | + | + | + | + | — | — | — | — |
| | 2 | + | + | + | + | — | — | — | — |
| | 4 | + | + | + | + | + | + | + | — |

+ = growth

The addition of 10 per cent horse serum to fresh egg, however, gave a mixture which supported growth. Thus, it can be seen that under these conditions growth increased from 1 in $10^5$ to one of 1 in $10^8$ after four days' incubation.

(b) *Growth in whole (8-day incubated, fertile) egg.* As a result of these findings it was thought that the essential growth factor was absent from infertile eggs but was produced as the embryo developed its own circulatory system. Experiments did not confirm this since, as may be seen from Table II, fertile egg is also bactericidal.

TABLE II

*Growth in whole (fertile) egg*

| Days seeded mixture incubated | Mixture diluted 1 in: | | | | |
|---|---|---|---|---|---|
| | $10^2$ | $10^3$ | $10^4$ | $10^5$ | $10^6$ |
| 0 | + | + | + | + | — |
| 2 | + | + | + | — | — |
| 4 | + | — | — | — | — |
| 6 | — | — | — | — | — |

(c) *Growth in egg constituents.* Yolk and albumen were then tested separately. As may be seen from the results shown in Table III, while yolk did not support growth under these conditions it was evident that the bactericidal action of whole egg is largely resident in the albumen fraction.

TABLE III

*Growth in yolk and albumen*

| Medium | Days seeded medium incubated | Medium diluted 1 in: | | | |
|---|---|---|---|---|---|
| | | 10 | $10^2$ | $10^3$ | $10^4$ |
| Yolk | 0 | . | + | + | + |
| | 2 | . | + | + | + |
| | 4 | . | + | + | — |
| Albumen | 0 | + | + | + | + |
| | 2 | — | — | — | — |
| | 4 | — | — | — | — |

It has since been shown that yolk contains a growth factor which, in 10 per cent amounts, can replace serum* and that albumen from any egg (fertile or infertile), and even if 10 per cent serum is added, is bactericidal.

This bactericidal action of albumen, however, is not so marked as might be expected. The albumen requires to be present in relatively high concentrations to be effective. In one experiment showing this, various mixtures of albumen and plain broth were seeded with 1 ml. undiluted serum broth culture, loopful subcultures being made to serum broth at intervals thereafter. It was found (see Table IV) that 90 per cent albumen destroyed the organisms in under four days at 37° C., 70 per cent in six days, 50 per cent in less than 20 days and that lower concentrations showed no action up to the 20th day.

* I am indebted to Mr. R. W. White for this information.

TABLE IV

*The bactericidal action of albumen*

| Mixture (mls.) | | Days seeded mixture incubated before sub-culture | | | | | |
|---|---|---|---|---|---|---|---|
| Albumen | Broth | 0 | 2 | 4 | 6 | 10 | 20 |
| 9 | — | + | + | — | — | — | — |
| 8 | 1 | + | + | — | — | — | — |
| 7 | 2 | + | + | + | — | — | — |
| 6 | 3 | + | + | + | + | — | — |
| 5 | 4 | + | + | + | + | + | — |
| 4 | 5 | + | + | + | + | + | + |

It is perhaps worth recording that both virulent and non-virulent organisms appear to be equally susceptible to this action.

### Discussion

The observations recorded here do not shed much light on the failure to confirm the work of Sheriff and Piercy on attenuation but they *may* offer some explanation of the failure of avianised vaccine. Vaccine was prepared from whole egg or from egg minus yolk but including albumen and this is bactericidal. It is a possibility that the bactericidal action is due to lysozyme, which is known to be present in high concentration in albumen.

### Summary

1. The pleuropneumonia organism does not survive when mixed with the beaten up contents of fertile eggs.

2. The bactericidal action is a property of the albumen and not of the yolk.

### Acknowledgment

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# STUDIES ON HÆMORRHAGIC SEPTICÆMIA OF CATTLE
## II. The Detection of Naturally Acquired Immunity

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### Introduction

In the first paper in this series a description was given of naturally acquired immunity in Siamese buffaloes to *Pasteurella multocida* type I of Roberts. A simple tube agglutination test has been devised for the detection of immune animals and eliminate them from vaccination experiments. Slide agglutination tests were unsatisfactory. The test, although it seems elementary, is described in some detail as it depends on the relative inagglutinability of *P. multocida*

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type I in the first few subcultivations after primary isolation. This permits the test to be done on a single tube of undiluted serum. The test cannot be used for the other Roberts serotypes of *P. multocida* (II, III and IV) as in the equivalent growth phase these contain hyaluronic acid in the capsules and are inagglutinable (Bain, unpublished experiments). However, it is becoming clear that the type I is the main if not the exclusive cause of true hæmorrhagic septicæmia of cattle in South-East Asia and elsewhere (Hudson, 1954; Bain, 1954 a and b).

### Description of the Test

A virulent strain of *P. multocida* type I Roberts is selected. This should be capable of killing cattle or buffaloes in a dose of 1 ml. of a $10^{-5}$ dilution of a six-hour broth culture injected subcutaneously. The work reported herein has been done entirely with one strain, "Insein," isolated in Burma, with initial checks against two other strains. [Dried cultures of "Insein" can be supplied on request to the author.] Blood taken from an experimentally infected ox shortly before death is stored in the deep-freezer at —20° C.; this keeps for months and serves as a suitable starting material. In the domestic refrigerator the bacteria may die in six weeks. The blood is cultured on to agar plates, which are incubated overnight; suitable colonies are picked into tubes of broth which are incubated for six hours and then used to inoculate Roux flasks or petri dishes of agar. After eighteen hours' growth the bacteria are washed off each flask with 5 ml. of 0.95 per cent saline and checked for purity by microscopic examination. There should be very little pleomorphism. The suspension is centrifuged at 1,000 r.p.m. for one minute to throw down coarse lumps and particles of agar. The supernatant is transferred by pipette into another tube and centrifuged at high speed for twenty minutes. This supernatant is discarded and the packed cells resuspended in a small volume of saline. The suspension is standardised for density at four times that of Brown's opacity tube No. 6. If the suspension is to be kept for a few days it is best to store the centrifuged cells in the refrigerator with very little saline over them. Preservatives should not be added. Formalin in an actual strength of 0.25 per cent in the suspension is strongly inhibitory to the agglutination reaction. Phenol 0.5 per cent leads to erratic results. A promising modification not yet fully tested is the addition of 100,000 units of crystalline penicillin G to each 10 ml. of suspension. Heating the bacteria at 60° C. for thirty minutes destroys the capsules and renders the cells highly agglutinable.

The medium on which the bacteria are grown is very important. The following formula is satisfactory.

| | |
|---|---|
| Difco Bacto-peptone ... | 10 gms. |
| Difco Yeast Extract ... | 2.5 gms. |
| Glucose ... ... ... | 5 gms. |
| Sodium chloride ... | 5 gms. |
| Agar powder ... ... | 15 gms. |
| Meat infusion ... ... | 1,000 ml. |

*pH* 7.4

The test is a simple tube agglutination one, using undiluted serum and live bacterial suspension in a 5 to 1 ratio. As no preservatives are used aseptic precautions are necessary. The blood samples should be collected from the jugular in sterile tubes; the agglutination tubes and the saline should be sterilised. The agglutination tubes need not be plugged. After samples of clotted blood have been spun lightly in the centrifuge, ten drops of the clarified serum from each are transferred to agglutination tubes with a simple dropping pipette. Two drops of bacterial suspension are added to each tube from the same pipette or one with a similar orifice. The rack is shaken and placed in a 37° C. incubator for ten minutes. It is then removed, shaken again for half a minute and replaced in the incubator for five minutes. Finally the rack is removed, shaken once more, and left at room temperature. The following morning it is shaken again and left for six hours before reading. If the shakings are omitted a few positives may be missed.

The results with buffalo sera described in this paper were obtained in this way. It has since been found that in ox sera pasteurella multiply more readily and a clearer result is obtained by holding the racks not at room temperature but in the refrigerator after the initial brief incubation. When penicillin is included in the test the racks may be left at room temperature.

The reading of the test is fairly orthodox. Complete sedimentation and complete clearing is awarded ++++; definite but not complete clearing with a substantial deposit +++; without definite clearing of the supernatant ++, + or ± depending on the amount of deposit. A few difficult judgments arise between ++ and +++; these have been called ++± and such tests are always repeated in triplicate. This reading scale is admittedly subjective but experience has shown it to be highly reproducible with the same or different people checking the tests.

When the tubes have been incubated and shaken, strong agglutination is apparent in most sera but the reaction bears little relationship to actual immunity as determined by challenge. This early reaction appears to be caused by conglutinin acting in concert with complement. It is abolished in all but strongly reacting or hyperimmune sera by heating the sera at 56° C. for thirty minutes before the test. Addition of fresh guinea-pig complement to the inactivated sera restores the agglutinating power to about 75 per cent of its former level. In a large batch of sera a few are always found which give a +++ agglutination at 20 minutes but later revert to negative. A few may be negative at 20 minutes but slowly become ++++ within 24 hours.

With bacteria showing the correct level of agglutinability, the serum agglutinins do not display a high titre. In some strongly reacting sera agglutination may continue to occur in one in four or even one in eight dilutions but in some animals known to be immune the reaction fades abruptly with dilution and may only show up in undiluted serum.

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### Correlation between Test and Challenge

In Table I is set out the results of the serum agglutination test on buffaloes and the result of challenge with approximately 50,000 L.D. of *P. multocida* type I in the form of 0.5 ml. of a six-hour broth culture injected subcutaneously in the neck.

**TABLE I**

*Final Reading of Agglutination Test and Result of Challenge*

| Buffalo No. | Reaction | Challenge | Buffalo No. | Reaction | Challenge |
|---|---|---|---|---|---|
| 55 | ++++ | survived | 81 | +++ | survived |
| 78 | ++++ | survived | 117 | +++ | survived |
| 80 | ++++ | survived | 119 | +++ | survived |
| 89 | ++++ | survived | 123 | +++ | survived |
| 97 | ++++ | survived | 150 | +++ | survived |
| 118 | ++++ | survived | 26 | ++± | survived |
| 124 | ++++ | survived | 122 | ++± | survived |
| 127 | ++++ | survived | 67 | ++± | died |
| 140 | ++++ | survived | 102 | ++± | died |
| 141 | ++++ | survived | 49 | ++ | died |
| 143 | ++++ | survived | 62 | ++ | died |
| 182 | ++++ | survived | 77 | ++ | died |
| 184 | ++++ | survived | 88 | ++ | died |
| 186 | ++++ | survived | 57 | + | died |
| 24 | ++++ | survived | 82 | + | died |
| 72 | ++++ | survived | 86 | + | died |
| 181 | +++ | survived | 94 | + | died |
| 28 | +++ | survived | 185 | + | died |
| 33 | +++ | survived | 98 | − | died |
| 44 | +++ | survived | 99 | − | died |
| 74 | +++ | survived | 85 | − | died |
| 75 | +++ | survived | | | |

In the agglutination test buffaloes 85, 94 and 99 all showed an early ++++ reaction which later reverted to negative or one plus; they showed no signs of immunity when challenged. Buffalo 141 was entirely negative at 20 minutes but the test came down slowly to ++++ at the final reading and it was immune when challenged.

The test has been applied to 20 other buffaloes in current experiments and in every instance there has been complete agreement between the serum agglutination reaction and the result of challenge with doses ranging from approximately 500 to 25,000 L.D.

In Australia true hæmorrhagic septicæmia of oxen is unknown, as *P. multocida* type I has not yet been isolated. The agglutination test described above was applied to the sera of 200 cattle taken at random but none was ++±

or more. Twenty-eight showed reactions up to +++, indicating that some low-level reactions are non-specific, as might be expected in a test of this nature.

### Antigens of "P. multocida" Type I

As a working hypothesis Bain (1954 a) postulated the existence of an antigen analogous to the Vi antigen of *Salmonella typhi*. The suggestive evidence has been relative inagglutinability of cultures soon after primary isolation, the greater agglutinability of these cells in cetyl-trimethyl ammonium bromide (CTAB), and the presence of complement fixing antibodies in the sera of cattle immunised with these so-called "phase I" cells. It had been already found that *P. multocida* types II, III and IV are readily decapsulated by hyaluronidase and rendered agglutinable in homologous antisera. Type I strains resist the direct action of hyaluronidase on the intact cells at *pH's* from 4.5 to 9.5. (A strain of unknown type has been found to be decapsulated by the enzyme only at *pH* of 9.5 in M/200 carbonate-bicarbonate buffer although hyaluronic acid was isolated from extracted capsular polysaccharide by precipitation with CTAB.) The properties of serum agglutinability and CTAB agglutinability are consistent with the presence of hyaluronic acid. Carter and Annau (1953) have found polysaccharide from a bovine type I strain to be depolymerised by bull testis hyaluronidase. Polysaccharides obtained from "phase I" cells of "Insein" strain are not depolymerised by bull testis hyaluronidase as measured by the turbidimetric method of Kass and Seastone (1944).

In agar diffusion columns with a modification of the technique of Oudin, extracts of the capsules of type I strains show six bands indicating antigenic complexity. With small variations in time and intensity similar patterns have been obtained with five variants from the "Insein" strain. Absorption experiments with immune rabbit sera and these variants also suggest that the antigenic differences are more quantitative than qualitative. Carter and Annau (loc. cit.) comment on a similar suggestion in their findings. For the purpose of the test described in this paper, cells obtained from the first subculture of a freshly passaged strain such as "Insein" on the appropriate medium give reproducible results.

### Discussion

This simple test has clarified the difficulties concerning experiments on hæmorrhagic septicæmia in Thailand. It has not failed on some seventy buffaloes tested. A weakness of the test is the lack of distinction between specific and non-specific low-level reactors. An animal which has an existing slight specific immunity would react better to vaccination and would tend to flatter an indifferent vaccine. The test is simple and reproducible. A multi-dilution test with a more agglutinable suspension would need to be calibrated by actual challenge experiments every time as suitable suspensions vary so much in agglutinability.

The test is not so suitable for assessing the immunity of vaccinated animals because of the occurrence in them of blocking antibodies. This is dealt with in

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a later paper in this series. No explanation can be offered at present for the difference between vaccinated animals and those acquiring immunity naturally.

**Summary**

A simple agglutination test is described for the detection of cattle with naturally acquired immunity to hæmorrhagic septicæmia. The application of the test to experimental buffaloes purchased in Thailand has enabled vaccination experiments to be conducted with some confidence.

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# *REVIEWS*

THE BLOODSTOCK BREEDERS' REVIEW. Vol. XLII, 1953. Founded, Edited and Published by The British Bloodstock Agency, Ltd., 26 Charing Cross Road, London, W.C.2.

THIS annual authoritative encyclopædia is to hand and in a from worthy of Coronation Year. The wealth of information contained within its pages is stupendous, and the task of compiling, analysing and classifying the material must be a prodigious one, and judging by the increase in volume since the lean years of the war period the editors deserve our commiseration, congratulations and thanks. The format follows the plan of previous issues, the illustrations in themselves warrant the purchase of the book, the text and statistical tables will be found to be intriguing and to contain a wealth of information of great value to all concerned with the thoroughbred industry. All this is enclosed in a binding that will grace the shelves of the most æsthetic bibliophile.

PORPHYRINS, by A. Vannotti (translated by C. Rimington). London: Hilger and Watts, Ltd., 1954. Pp. 258, plates 15. Price 50s.

THE more specialised the subject of a scientific monograph the greater the difficulty there is in reviewing it, for the number of people with training sufficient to assess the accuracy of such monographs is usually so small that book production

for them alone would be uneconomic. In this instance, therefore, the review is largely an assessment of the value of the book to a wider public having some interest, though not a paramount one, in the porphyrins.

At the outset it must be said that the veterinary content of the book is small, even allowing for the statement that "metabolism . . . in mammals . . . is usually similar to that in man." In fact, only chapter VI, dealing with subjects such as the action of porphyrins on photosensitivity, on the circulatory system and on the intestines, can it be said that the section is of present veterinary application, even though the porphyrins, those integral components of pigments like hæmoglobin and chlorophyll, are so important biologically.

As a reference book and as one which may suggest parallels between human and animal metabolism, this publication undoubtedly has value. It deals with the physico-chemical properties of porphyrins (nine tables of constants), methods for porphyrin extraction, the general biology of the pigments and their metabolism, as well as porphyria and the treatment of porphyrin diseases.

Of the adverse criticisms to be made, probably the majority are not the responsibility of author or translator. The references, for example, of which there are some 500, suggest that the book gives a good account of its subject up to about 1951. The translator's preface is dated 1952 whilst the date of publication is 1954. This is too slow for such a monograph. Furthermore, although the binding and printing are good, the drab yellow paper used befits neither the work nor the price of the volume.

For the inadequate index of one and a half pages, with a meagre 103 entries for 250 pages of text, presumably the author must bear some responsibility: the non-specialist needs better guidance. Apart from a possible saving of cost, the reviewer sees little to commend and much to annoy, in grouping all references under chapter headings at the end of the book.

The translation appears good, whilst only one minor error was noticed, namely, that reference 35b on page 51 is not in the book at all.

ARTIFICIAL BREEDING AND LIVESTOCK IMPROVEMENT, by G. W. Stamm. Pp. 304. Chicago and New York: Windsor Press, 1954. Price $3.50.

THE writing of a "popular" work dealing with a technical subject can never be an easy task. "Artificial Breeding and Livestock Improvement" is an American attempt to describe for the benefit of the cattle owner the purpose and techniques of artificial insemination and methods of improving livestock by selection and breeding. In addition, the author discusses the technique of pregnancy diagnosis, and procedures for delivering a calf in the case of difficult parturition. The breeds of livestock in the United States are given in the concluding chapters.

The book is an interesting and up-to-date production which is written in an informal style. The chapters on the physiology of reproduction and the artificial insemination of cattle are a concise and clear exposition of the subject:

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the same can be said for the chapter devoted to breeding problems. The description of the procedures for diagnosing pregnancy and dealing with difficult calving cases seem out of place in a book of this nature, particularly as the danger of a novice dealing with either of these matters is not properly stressed.

Methods that can be used by the stock breeder for improving his livestock are explained very clearly. It is good to see that full attention is given to the selection of cattle for breeding purposes.

The book is copiously illustrated, but many of the pictures tend to be irrelevant, either to the captions, or even to the book itself. There are several minor inaccuracies in the text, but these are not serious. In general, the book should prove to be of use and interest to the stockman.

BEES ARE WEALTH, by Dr. I. Mann, the Veterinary Services, Kenya. Nairobi: Eagle Press. Price 1s.

THE aim of this book is to popularise bee-keeping in Kenya and to improve the quality of the local honey and beeswax so that these commodities may take their proper place as one of the country's important exports.

Written with the greatest lucidity and simplicity and clearly illustrated, the book is very good in every way. In fifty pages or so of text, the author describes the whole art of bee-keeping and dispels every vestige of mystery which, to the uninitiated, has always shrouded this department of animal husbandry. Bearing constantly in mind that the object of the book is to make bee-keeping attractive to the thousands, great pains are taken to describe how hives, protective clothing and all the other appliances may be improvised out of cheap and ready-to-hand materials. The sections on the collection of the wax and honey are done very well and it is a book which should find a much wider circulation than the one for which it is intended.

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## *NOTICES*

IMPERIAL CHEMICALS (PHARMACEUTICALS), LTD., have announced the introduction of "Hibitane," an antibacterial compound. It is at present available to the veterinary profession in three preparations. Intramammary Cream for Bovine Mastitis, Obstetric Cream, and Pessaries indicated for treatment of Metritis and Endometritis. It is expected that further preparations will shortly be announced as the result of widespread clinical trials. Further particulars may be obtained from the manufacturers.

LEDERLE LABORATORIES, LTD., announce the availability of Veterinary Aureomycin Crystalline Capsules, Veterinary Aureomycin Crystalline Ointment for Udder Infusion and Veterinary Aureomycin Crystalline Ophthalmic Ointment. Other forms are in production and will be announced in due course.

GLAXO LABORATORIES, LTD., announce the introduction from November 8 of a 540 c.c. bottle of Veterinary Intradex (6 per cent dextran in normal saline). This size is in addition to the 125 c.c. bottle previously available.